Disclosed is a transgenic knockout mouse whose genome has a homozygous disruption in its endogenous Gpx1 and Gpx2 genes, wherein the disruptions result in a decrease in GPX activity in the transgenic mice when compared to non transgenic mice of the same type. Methods for production of the mouse are presented. Also disclosed are cells derived from the transgenic knockout mouse. The invention further provides a mouse model for the disorders of ileitis, colitis, inflammatory bowel disease, ileal cancer and myeloleukemia. The mouse can be used in a method for identifying therapeutic agents for the treatment of an individual diagnosed with one or more of said disorders.
DKOs with and without tumors as a function of age

number of mice

age in months

- no tumor
- tumors

5 month: 5/16 (Tumor/Total)
6-8 month: 1/5
9-13 month: 10/21
Location of tumors in SB (fraction of SB from stomach to cecum)
MICE WITH COMBINED DISRUPTION OF GPX1 AND GPX2 GENES HAVE GROWTH RETARDATION, HYPOTHERMIA, AND COLITIS AND PROVIDE A MOUSE MODEL FOR CANCER

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application is a Continuation-in-part of application No. 09/973,196, which is related to provisional application No.60/238,443.

[0002] The publications and other materials used herein to illuminate the background of the invention or provide additional details respecting the practice are incorporated by reference.

TECHNICAL FIELD OF THE INVENTION

[0003] The present invention is directed towards specific double knockout (DKO) mice and their use as animal models. More specifically, the double knockout animals contain a disruption in the genes encoding GPX-1 and GPX-2/1. Corresponding cells which are amenable to tissue culture are also part of the invention, as are methods of using such cells, including their use as a tool for identifying therapeutic agents. In addition, the invention is directed towards a mouse model of cancer of the small bowel.

BACKGROUND OF THE INVENTION


[0006] Depending on the type of insult, lack of GPX activity can also be beneficial under certain circumstances. Mice overexpressing GPX-1 and GPX-2 have low levels of peroxidases of prostate gland and are more sensitive to hyperthermia (Mirochnitchenko, O., Palnikar, U., Philbert, M. & Inouye, M. (1995) Proc. Natl. Acad. Sci. USA 92, 8124-6). We have found that the jejunal crypt of Gpx1-KO mice regenerates better than that in the wildtype after exposure to high dose γ-irradiation (Esworthy, R. S., Mann, J. R., Sam, M. & Chu, F. (2000) Am J Physiol Gastrointest. Liver Physiol 279, G426). Perhaps the higher level of GPX-1 in the Gpx1-KO mouse intestine are directly responsible for crypt regeneration. Furthermore, the lack of GPX activity in the Gpx1-KO mouse can be protective against kainic acid-induced limbic seizures and neurodegeneration (Jiang, D., Akopian, G., Ho, Y. S., Walsh, J. P. & Andersen, J. K. (2000) Exp. Neurol. 164, 257-68). This appears to result from decreased receptor function for N-methyl-D-aspartate (NMDA), since kainic acid induces NMDA-dependent seizure. Increased GPX activity in mice overexpressing the Gpx1 gene may have enhanced carcino-genic response in skin treated with 7,12-dimethylbenz[a] anthracene and 2-0-tetradecanoylphorbol-1-3-acetate (Lu, Y.
P., Lou, Y. R., Yen, P., Newmark, H. L., Nfirochnitchenko, O. L., Inouye, M. & Huang, M. T. (1997) Cancer Res. 57, 1468-74. The mechanism of the pro-carcinogenic activity is not known, but it is apparent that elevated antioxidant activity can be debilitating, depending on the type of insult.

[0007] GPX activity is also implicated in protection against infectious agents. For example, Jaeschke et al. have found that Gpx1-KO mice are more susceptible to neutrophil-mediated parenchymal cell injury during endotoxemia (Jaeschke, H., Ho, Y. S., Fisher, M. A., Lawson, J. A. & Farhood, A. (1999) Hepatology 29,443-50). The galactosamine/endotoxin induced acute liver failure involves neutrophils and GPX protects hepatocytes against peroxides generated by infiltrated neutrophils in the liver. It has also previously been shown that Gpx1-KO mice are more susceptible to coxsackievirus-induced myocarditis (Beck, M. A., Eseworthy, R. S., Ho, Y. S. & Chu, F. F. (1998) Faseb J. 12,1143-9). Viral antibody titers in the Gpx1-KO mice are less than 20% of those found in the wildtype mice, suggesting that cellular immune response is impaired in the Gpx1-KO mice.

[0008] Gpx2-KO mice have also recently been generated and these mice appear to be normal (Eseworthy et al., Am. J. Physiol. Gastrointest. Liver Physiol. 279, G426-G436). Unlike the Gpx1 gene, which is expressed ubiquitously, the Gpx2 gene is expressed specifically in epithelium. The Gpx2 gene is highly expressed in the gastrointestinal tract, and is also present in the breast, lung, and human liver (Chu, F. F., Doroshow, J. H. & Eseworthy, R. S. (1993) J. Biol. Chem. 268, 2571-6; Chu, F. F., Eseworthy, R. S., Lee, L. & Wilczynski, S. (1999) J. Nutr. 129, 1846-1854). In the GI-epithelium, GPX-1 and GPX-GI contribute to most of GPX activity (Eseworthy, R. S., Swiderek, K. M., Ho, Y. S. & Chu, F. F. (1998) Biochim. Biophys. Acta. 1381,213-26.). The lack of pathology in Gpx2-KO mice is not unexpected, since the Gpx2 gene has limited tissue expression, the Gpx1 gene is co-expressed in tissues expressing the Gpx2 gene, and Gpx1 and Gpx2-GI have similar biochemical and cellular properties.


[0010] The evaluation of chemical compounds for potential efficacy as human therapeutics necessitates data and information of a compound’s efficacy in vivo. Ideally, in vitro system would be human but ethical and pragramic reasons prevent such data from being accumulated. As an alternative, many laboratory animals provide satisfactory systems for screening potential therapeutics for treating human physiological disorders. Recent advances in recombinant DNA technology have enabled researchers to genetiically manipulate the genomes of animals to enhance such animal model systems. For example, the technique of transgenic generation have been utilized to produce knockout mice that do not express a particular endogenous gene.

[0011] There presently exists a need for animal models which can be utilized to study the physiological function of GPX activity in the GI-tract. One approach to generate a useful model for such studies would be double knockout (double-KO) mice with a combined disruption of both alleles of each of the Gpx1 and Gpx2 genes.

SUMMARY OF THE INVENTION

[0012] In accordance with the present invention, an animal model is provided for studying the significance of GPX-1 and GPX-GI, in particular with regard to how these two gene products interact in animal physiology.

[0013] In one aspect, the invention provides a transgenic animal deficient in both GPX-1 and GPX-GI activity. In a preferred embodiment, the animal is a mouse. The deficiency is a result of a homozygous double knockout of the Gpx1 and Gpx2 genes in said transgenic mouse.

[0014] In another aspect, the invention provides an animal model for the study of pathophysiological function of GPX activity in the ileum and colon in mammals. We have found that the homozygous double-KO mice of the present invention can exhibit symptoms associated with ileitis, colitis, growth retardation, hypothermia, wasting syndrome, inflammatory bowel disease, cancer in the lower GI-tract and leukemia.

[0015] In one aspect, the invention provides an animal model for the study of the degree of functional redundancy of GPX-1 and GPX-GI in the ileum and colon in mammals.

[0016] In one embodiment, the invention provides transgenic mice which have a homozygous knockout of the Gpx1 gene in said transgenic mice, together with a heterozygous knockout of one allele of the Gpx2 gene. The invention thus provides an animal model for the study of the degree of functional redundancy of GPX-1 and GPX-GI in the ileum and colon.

[0017] In another embodiment, the invention provides transgenic mice which have a homozygous knockout of the Gpx2 gene, together with a heterozygous knockout of one allele of the Gpx1 gene in said transgenic mice. The invention thus provides another animal model for the study of the degree of functional redundancy of GPX-1 and GPX-GI in the ileum and colon.
[0018] In another embodiment, the invention provides a transgenic double knockout mouse whose genome comprises a homozygous disruption of the endogenous Gpx1 gene and a homozygous disruption of the endogenous Gpx2 gene (a transgenic double knockout Gpx1/Gpx2 mouse), wherein each disruption comprises the insertion of a transgene, and wherein the combined disruptions result in a decreased level of GPX-1 and GPX-Gl production and decreased number of cells producing GPX-I and GPX-Gl in the transgenic mouse as compared to a nontransgenic mouse.

[0019] In another embodiment, the invention provides a transgenic double knockout Gpx1/Gpx2 mouse which exhibits a physiological disease, symptom or symptoms selected from the group consisting of ileitis, colitis, hyperthermia, decreased rate of weight gain, perianal ulceration, diarrhea, wasting syndrome, inflammatory bowel disease, cancer of the ileum and myeloleukemia.

[0020] In another embodiment, the invention provides a cell or cells isolated from any of the following: a double knockout Gpx1/Gpx2 transgenic mouse, a transgenic mouse having a heterozygous knockout of the Bpdx1 gene and a heterozygous knockout of the Gpx2 gene, a transgenic mouse having a homozygous knockout of the Gpx1 gene and a heterozygous knockout of one allele of the Gpx2 gene and a transgenic mouse having a homozygous knockout of the Gpx1 gene and a heterozygous knockout of one allele of the Gpx1 gene.

[0021] In one embodiment, the invention provides a transgenic double knockout Gpx1/Gpx2 mouse which further comprises a mouse which is germ free.

[0022] In another embodiment, the invention provides the double knockout of the Gpx1 and Gpx2 genes in mice having different genetic backgrounds. The invention thus provides means to identify other genes that affect the severity of ileitis, colitis, inflammatory bowel disease symptoms and progression to cancer.

[0023] In another embodiment, the invention provides the double knockout of the Gpx1 and Gpx2 genes in a genetic background of a B6 mouse.

[0024] In another aspect, the invention provides a method of selecting an agent for treating a metabolic disorder selected from the group consisting of: ileitis, colitis, hyperthermia, decreased rate of weight gain, perianal ulceration, diarrhea, wasting syndrome, inflammatory bowel disease, ileal cancer and myeloleukemia comprising:

(a) measuring a symptom in a knockout mouse whose genome is manipulated to comprise a homozygous disruption of both the endogenous Gpx1 and Gpx2 genes, wherein the disruption of both the Gpx1 and Gpx2 genes results in said knockout mouse exhibiting one of said disease, symptom or symptoms;

(b) administering an agent to said mouse;

(c) measuring one or more of said symptoms in the mouse after administering the agent; and

(d) comparing at least one of said symptoms in the mouse before and after administering the agent, wherein a decrease in said disease, symptom or symptoms after administering the agent indicates the agent is an agent for treating said disease, symptom or symptoms associated with a metabolic disorder.

[0029] In another embodiment, the method of observing the effects of treatment of a disease, symptom or symptoms in double-KO Gpx1 and Gpx2 transgenic mice by administering an agent is observed and compared in double-KO Gpx1 and Gpx2 mice having different genetic backgrounds. The method comprises:

(a) measuring a symptom in a first double knockout mouse having a first genetic background, whose genome is manipulated to comprise a homozygous disruption of both the endogenous Gpx1 and Gpx2 genes, wherein the disruption of both the Gpx1 and Gpx2 genes results in said knockout mouse exhibiting a disease, symptom or symptoms selected from the group consisting of: ileitis, colitis, hyperthermia, decreased rate of weight gain, perianal ulceration, diarrhea, wasting syndrome, inflammatory bowel disease, ileal cancer and myeloleukemia;

(b) measuring said symptom in a second double knockout mouse having a second genetic background, whose genome is manipulated to comprise a homozygous disruption of both the endogenous Gpx1 and Gpx2 genes, wherein the disruption of both the Gpx1 and Gpx2 genes results in said knockout mouse exhibiting at least one of said disease, symptom or symptoms;

(c) administering an agent to said first and second mouse;

(d) measuring one or more of said symptoms in the first and second mouse after administering the agent; and

(e) comparing at least one of said symptoms in said first and second mouse before and after administering the agent, wherein a decrease in said disease, symptom or symptoms after administering the agent indicates the agent is an agent for treating said disease, symptom or symptoms associated with a metabolic disorder.

[0035] In another embodiment, the invention provides a method of observing the effects of treatment of a disease, symptom or symptoms in single and Gpx1/Gpx2 double knockout transgenic mice in mice with a B6 genetic background or in hybrid mice having a $\frac{1}{2}$ B6, $\frac{1}{4}$ 129 SvJ and $\frac{1}{4}$ 129Sv genetic background.

[0036] In another embodiment, the invention provides a method of selecting an agent that modulates GPX enzyme activity comprising:

(a) administering an agent to a first group of isolated mouse intestinal epithelial cells and not to a second group of mouse intestinal epithelial cells, wherein the genome of both the first and second isolated mouse cell groups has been manipulated to comprise a homozygous disruption of both alleles of the endogenous Gpx1 gene and Gpx2 genes, and wherein the homozygous disruption of both the endogenous Gpx1 gene and Gpx2 genes prevents expression of functional GPX proteins; and

(b) determining the amount of GPX enzyme activity of the first and second cell groups, wherein a difference in the amount of proliferation of the first cell group as compared to the second cell group indicates that the agent modulates GPX enzyme activity. In another embodiment the
isolated cells can be observed for a change in level of expression of a marker associated with cancer.

[0039] In another embodiment, the invention provides a transgenic animal whose genome contains a homozygous disruption of both the endogenous Gpx1 gene and Gpx2 genes, wherein said animal develops cancer.

[0040] In another embodiment, the invention provides an animal model for the development of ileitis, colitis, inflammatory bowel disease, ileal cancer and or myeloleukemia. The model comprises a transgenic animal whose genome comprises a homozygous disruption of the endogenous Gpx1 gene and a homozygous disruption of the endogenous Gpx2 gene, wherein disruption of the Gpx1 and Gpx2 genes is sufficient to effect one or more signs or symptoms in the animal associated with ileitis, colitis, inflammatory bowel disease, ileal cancer and or myeloleukemia.

[0041] The invention further provides a method to screen for potential therapeutic agents for the treatment of ileitis, colitis, inflammatory bowel disease, ileal cancer and or myeloleukemia. The method comprises the steps of: a) administering a potential therapeutic agent to a first transgenic animal whose genome comprises a homozygous disruption of both the endogenous Gpx1 gene and Gpx2 genes; b) maintaining the animal for a time sufficient to permit the detection of a change in one or more signs or symptoms in the animal associated with ileitis, colitis, inflammatory bowel disease, ileal cancer and or myeloleukemia; c) observing the animal for a change in at least one sign or symptom associated with ileitis, colitis, inflammatory bowel disease, ileal cancer and or myeloleukemia, wherein a second transgenic animal having the same genetic background as the first transgenic animal and whose genome also comprises a homozygous disruption of both the endogenous Gpx1 gene and Gpx2 genes has been maintained under the same conditions as the first animal but has not received the potential therapeutic agent; and d) determining whether one or more signs or symptoms associated with ileitis, colitis, inflammatory bowel disease, ileal cancer and or myeloleukemia is present in the second transgenic animal but not in the first transgenic animal; wherein a potential therapeutic agent will be one that causes a lower incidence of at least one sign or symptom associated with ileitis, colitis, inflammatory bowel disease, ileal cancer and or myeloleukemia in the first transgenic animal.

[0042] The invention further provides a method to screen for potential therapeutic agents for the treatment of ileitis, colitis, inflammatory bowel disease, ileal cancer and or myeloleukemia. The method comprises the steps of: a) administering a potential therapeutic agent to an isolated first cell from a first transgenic animal whose genome comprises a homozygous disruption of both the endogenous Gpx1 gene and Gpx2 genes; b) maintaining the cell for a time sufficient to permit the detection of a change in one or more signs or symptoms in the first cell associated with ileitis, colitis, inflammatory bowel disease, ileal cancer and or myeloleukemia in the first cell; c) observing the first cell for a change in at least one sign or symptom associated with ileitis, colitis, inflammatory bowel disease, ileal cancer and or myeloleukemia, wherein a second cell from a second transgenic animal having the same genetic background as the first transgenic animal and whose genome also comprises a homozygous disruption of both the endogenous Gpx1 gene and Gpx2 genes has been maintained under the same conditions as the first cell but has not been exposed to the potential therapeutic agent; and d) determining whether one or more signs or symptoms associated with ileitis, colitis, inflammatory bowel disease, ileal cancer and or myeloleukemia is present in the second transgenic cell but not in the first transgenic cell; wherein a potential therapeutic agent will be one that causes a lower incidence of at least one sign or symptom associated with ileitis, colitis, inflammatory bowel disease, ileal cancer and or myeloleukemia in the first transgenic cell.

[0043] The invention further provides a method for assessing the therapeutic effect of a heterologous gene of interest on the development of ileitis, colitis, inflammatory bowel disease, ileal cancer and or myeloleukemia, which comprises the steps of: expressing a heterologous gene of interest in a first transgenic animal comprising a homozygous disruption of both the endogenous Gpx1 gene and Gpx2 genes; maintaining the first transgenic animal for a time sufficient to permit the detection of one or more signs or symptoms in the first transgenic animal associated with ileitis, colitis, inflammatory bowel disease, ileal cancer and or myeloleukemia in the first transgenic animal; observing the first transgenic animal for a change in at least one sign or symptom associated with ileitis, colitis, inflammatory bowel disease, ileal cancer and or myeloleukemia, wherein a second transgenic animal comprising a homozygous disruption of both the endogenous Gpx1 gene and Gpx2 genes does not express the gene of interest, wherein the second transgenic animal has been maintained under the same conditions as the first transgenic animal; and determining whether one or more signs or symptoms associated with ileitis, colitis, inflammatory bowel disease, ileal cancer and or myeloleukemia is present in the second animal, wherein a gene of interest which reduces the sign or symptom will be one that causes a lower incidence of at least one sign or symptom associated with ileitis, colitis, inflammatory bowel disease, ileal cancer and or myeloleukemia in the first animal.

[0044] The invention further provides a method of identifying one or more marker genes or proteins associated with ileal cancer and or myeloleukemia, which comprises the steps of: expressing the marker gene or protein in a first transgenic animal whose genome comprises a homozygous disruption of both the endogenous Gpx1 gene and Gpx2 genes; maintaining the first transgenic animal for a time sufficient to permit the detection a change in one or more signs or symptoms in the first transgenic animal associated with ileitis, colitis, inflammatory bowel disease, ileal cancer and or myeloleukemia, wherein a second transgenic animal comprising a homozygous disruption of both the endogenous Gpx1 gene and Gpx2 genes does not express the marker gene or protein, wherein the second transgenic animal has been maintained under the same conditions as the first transgenic animal; and determining whether one or more signs or symptoms associated with ileitis, colitis, inflammatory bowel disease, ileal cancer and or myeloleukemia is present in the first transgenic animal, wherein a marker gene or protein associated with ileitis, colitis, inflammatory bowel disease, ileal cancer and or myeloleukemia will be one that causes a higher
incidence of at least one sign or symptom associated with ileitis, colitis, inflammatory bowel disease, ileal cancer and or myeloleukemia in the first animal.

[0045] In particularly preferred embodiments, the first and second transgenic animals utilized in the methods of the invention are mice.

BRIEF DESCRIPTION OF THE FIGURES

[0046] FIG. 1A depicts the results of Southern analysis of various Gpx1 and Gpx2 genes in knockout mice.

[0047] FIG. 1B depicts the results of GPX activity in Gpx1 and Gpx2 double knockout mice compared to non double-KO littersmates.

[0048] FIG. 2A is a graphical representation of the growth (in body weight) of Gpx1 and Gpx2 homozygous double-KO mice compared with their non double-KO littersmates.

[0049] FIG. 2B is a graphical representation of the age at which Gpx1 and Gpx2 homozygous double-KO mice show growth retardation.

[0050] FIG. 3A is a graphical representation of body temperature of Gpx1 and Gpx2 homozygous double-KO mice compared to non-double-KO littersmates.

[0051] FIG. 3B is a graphical representation of body temperature in response to stress of Gpx1 and Gpx2 homozygous double-KO mice compared to non-double-KO littersmates.

[0052] FIG. 4 is a photograph of histological preparations of Gpx1 and Gpx2 homozygous double-KO mice compared to non-double-KO littersmates.

[0053] FIG. 5 is a graphical representation of the incidence of tumors in Gpx1 and Gpx2 homozygous double-KO mice as a function of age.

[0054] FIG. 6 is a graphical representation of the relative location of tumors in Gpx1 and Gpx2 homozygous double-KO mice.

[0055] FIG. 7 is a photographic representation of the location of tumors in the small intestine of a Gpx1 and Gpx2 homozygous double-KO mouse.

DETAILED DESCRIPTION OF THE INVENTION

[0056] The invention provides a transgenic animal whose genome contains a homozygous disruption of both the endogenous Gpx1 gene and Gpx2 genes wherein said animal develops a sign or symptom selected from the group consisting ileitis, colitis, hypothermia, decreased rate of weight gain, perianal ulceration, diarrhea, wasting syndrome, inflammatory bowel disease, ileal cancer and myeloleukemia. In a preferred embodiment, the transgenic animal is a mouse. In another preferred embodiment, the invention provides a model for a sign or symptom associated with ileitis, colitis, hypothermia, decreased rate of weight gain, perianal ulceration, diarrhea, wasting syndrome, inflammatory bowel disease, ileal cancer and myeloleukemia. The transgenic mouse can for example have a genetic background selected from the group consisting of a B6 mouse, a C57B16/J hybrid and a 129sv/J hybrid mouse. Using techniques well known in the art and described elsewhere (Esoworthy et al. (2000) Am J Physiol Gastrointest Liver Physiol 279, G426-G436), a hybrid mouse having a homozygous disruption in a gene or genes can be maintained by inbreeding. Alternatively, hybrid mice having a homozygous or heterozygous disruption in a gene or genes are bred with one of the hybrid's parent strains to generate a subline wherein the mice have a homozygous disruption in a gene or genes in a genetic background for one or the other of the parent strains. In general, generation and maintenance of Gpx1 and Gpx2 homozygous double-KO mice was performed as described in Esoworthy et al, Am. J. Physiol.Gastrointest. Liver Physiol. 281:G848-855 (2001).

[0057] In a preferred embodiment, the double KO mice of the invention are % B6, % 129sv/J and % 129Sv/J. B6 and 129sv/J hybrids having a homozygous disruption of the Gpx1 gene are crossed to B6 and 129Sv/J hybrids having a homozygous disruption of the Gpx2 gene to produce Gpx1 and Gpx2 homozygous double-KO mice that are % B6, % 129sv/J and % 129Sv/J.

[0058] C57BL/6J(B6) and 129sv/J hybrids mice as described previously (Ho, et al. J. Biol. Chem. 272, 16644-51(1997)) having a Gpx1 knockout were backcrossed to B6 mice for 7 generations, producing mice greater than 90% B6 in genetic background. B6 and 129Sv/J hybrids (Esoworthy et al. Am. J. Physiol. Gastrointest. Liver Physiol. 279, G426-G436(2000)) having a Gpx2 knockout were also backcrossed to B6 mice for 7 generations. The resulting Gpx1 gene and Gpx2 knockout mice in a B6 background were crossed to produce double knockout mice in a greater than 90% B6 background. Two resultant double KO B6 mice had ileitis and colitis.

[0059] The transgenic animal of the invention provides an animal model for ileal cancer and or myeloleukemia. In a preferred embodiment, the model comprises a transgenic mouse whose genome contains a homozygous disruption of both the endogenous Gpx1 gene and Gpx2 genes wherein said animal develops one or more signs or symptoms of cancer of ileal cancer and or myeloleukemia. The transgenic mouse of the invention displays at least one sign or symptom associated with cancer is selected from the group consisting of ileitis, colitis, hypothermia, decreased rate of weight gain, perianal ulceration, diarrhea, wasting syndrome, inflammatory bowel disease, one or more tumors in the small bowel and myeloleukemia. In one embodiment, the transgenic mouse of the invention can further comprise a mouse which is a germ free mouse.

[0060] In another embodiment, the invention provides a method to screen for potential therapeutic agents for the treatment of ileal cancer and or myeloleukemia. A potential therapeutic agent is administered to a first transgenic animal whose genome comprises a homozygous disruption of both the endogenous Gpx1 gene and Gpx2 genes. The first transgenic animal is maintained for a time sufficient to permit the detection of a change in one or more signs or symptoms of ileal cancer and or myeloleukemia in the transgenic animal. A second transgenic animal having the same genetic background as the first transgenic animal and whose genome also comprises a homozygous disruption of both the endogenous Gpx1 gene and Gpx2 genes is maintained under the same conditions as the first animal but does not receive the potential therapeutic agent. The first and second animals are observed for a change in at least one sign
or symptom associated with ileal cancer and myeloleukemia. A therapeutic agent which prevents one or more signs or symptoms of ileal cancer and myeloleukemia in the first transgenic animal when compared to the second transgenic animal will be a potential therapeutic agent for the treatment or prevention of ileal cancer and myeloleukemia. In a preferred embodiment, the sign or symptom of ileal cancer is the development of tumors in the distal two thirds of the small intestine. In a preferred embodiment, detection of tumors in the small bowel of the animal is performed by sacrificing the first and second animal after a time sufficient for the detection of at least one tumor of the small bowel in the first and second animals has elapsed and observing the tissue of the small bowel using techniques well known in the art. In another preferred embodiment, the transgenic animal is a mouse. In one embodiment, the mouse has a B6 genetic background. In yet another preferred embodiment, the mouse has a genetic background of ½ B6, ¼ 129Sv/J and ¼ 129S3.

[0061] In another embodiment, a potential therapeutic agent is administered to a first transgenic animal whose genome comprises a homozygous disruption of both the endogenous Gp1 gene and Gp2 genes and to a second transgenic animal having a different genetic background from the first transgenic animal and whose genome also comprises a homozygous disruption of both the endogenous Gp1 gene and Gp2 genes and the effects of the therapeutic agent on the development of ileal cancer and myeloleukemia is compared in the two animals.

[0062] Many signs or symptoms associated with ileal cancer and myeloleukemia are known in the art which may be used to screen for the development of ileal cancer or myeloleukemia. Some non-limiting examples include ileitis, colitis, inflammatory bowel disease, the appearance of areas of dysplasia in epithelial cells of the small intestine, tumors in the small intestine, the presence of abnormal telomerase activity in cells, exfoliated cancer cells in stool and the presence of antibody to small intestine mucin antigen. Thus, in some embodiments, the animals do not have to be sacrificed to detect a sign or symptom associated with cancer.

[0063] It is contemplated that other methods of detecting the tumors or other signs or symptoms of cancer can be substituted for microscopic examination of tissue without departing from the scope of the invention. Non limiting examples may include withdrawing a body fluid from the first and second animal and analyzing the body fluid such as blood, for example, for the presence of one or more signs or symptoms of cancer of the ileum. By way of example, exfoliated cancer cells in stool or blood samples could be examined for reaction with antibody for small intestine mucin antigen or by PCR telomerase reactions, as known by those skilled in the art. See, e.g., Pinczower, et al., International J. Cancer 54(3) 391-396 (1993); Guadagni et al., Cancer Res. 56(22) 5293-5299; and Gauthier et al., Br. J. Cancer 84(5) 631-635 (2001).

[0064] The invention further provides a transgenic animal whose genome comprises a homozygous disruption of both the endogenous Gp1 gene and Gp2 genes wherein the animal’s genome can additionally comprise a DNA sequence encoding a heterologous gene of interest. The gene of interest may code for a biologically active nucleic acid or polypeptide including, for example, an immunomodulator, a peptide, an oligonucleotide and the like. The gene of interest can also be inserted within a target gene of the transgenic animal of the invention in order to disrupt that target gene, thereby generating a knockout mouse having a homozygous disruption of the Gp1, Gp2 and target genes. The heterologous gene of interest can comprise, for example, an antibiotic marker gene or an allelic variant of the gene to be disrupted, wherein the allelic variant is not expressed or is not biologically active. In addition, genes can be disrupted by providing an antisense RNA, a ribozyme and the like to prevent transcription or translation of the target gene.

[0065] The invention further provides a method for assessing the therapeutic effect of a heterologous gene of interest on the development of ileal cancer and myeloleukemia which comprises expressing the heterologous gene of interest in a first transgenic animal whose genome comprises a homozygous disruption of both the endogenous Gp1 gene and Gp2 genes. The first transgenic animal is maintained for a time sufficient to permit the detection a change in one or more signs or symptoms in the first transgenic animal associated with ileal cancer and myeloleukemia. Second transgenic animal having the same genetic background as the first transgenic animal and comprising a homozygous disruption of both the endogenous Gp1 gene and Gp2 genes which does not express the gene of interest is maintained under the same conditions as the first transgenic animal. Both transgenic animals are observed for the presence or absence of one or more signs or symptoms of ileal cancer and myeloleukemia.

[0066] In another embodiment, a heterologous gene of interest can be expressed in a first transgenic animal whose genome comprises a homozygous disruption of both the endogenous Gp1 gene and Gp2 genes. The heterologous gene of interest is not expressed in a second animal having a homozygous disruption in both the endogenous Gp1 gene and Gp2 genes but the first and second animals have different genetic backgrounds.

[0067] The invention further provides a method of identifying markers associated with ileitis, colitis, inflammatory bowel disease, ileal cancer and myeloleukemia, the method comprising comparing the presence, absence or level of expression of at least one gene or protein in a transgenic animal whose genome comprises a homozygous disruption of both the endogenous Gp1 gene and Gp2 genes. The heterologous gene of interest is not expressed in a second animal having a homozygous disruption in both the endogenous Gp1 gene and Gp2 genes but the first and second animals have different genetic backgrounds.

[0068] The invention further provides cells isolated from the knockout mice of the invention. Such cells can be of any cell type that can be isolated from the transgenic animal, utilizing techniques well known in the art. By way of example, isolated cells can include stem cells, epithelial cells, myofibroblasts and the like. The cells can be utilized
in in vitro experiments to study the physiologic characteristics of such cells and can comprise cell lines from the knockout mice.

[0069] General Methods

[0070] The practice of the present invention employs, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA, genetics, immunology, cell biology, cell culture and transgenic biology, which are known to one of ordinary skill in the related art.

[0071] Gpx1-KO mice were generated by using standard techniques as C57BL/6J (B6) and 129SvJ hybrids and B6 inbred mice as described previously (Ho, Y. S., Magenat, J. L., Bronson, R. T., Cao, J., Gargano, M., Sugawara, M. & Funk, C. D. (1997) J Biol Chem 272, 16644-51).

[0072] The generation of Gpx2-KO mice as B6 and 129SvJ hybrids and B6 mice has been described previously (Esworthy, R. S., Mann, J. R., Sam, M. & Chu, F. (2000) Am J Physiol Gastrointest Liver Physiol 279, G426-G436). These mice were housed in ventilated cage racks (Allentown Caging Equipment, Allentown, N.J.) under conventional housing conditions. The colony was monitored for infectious pathogens via sentinel mouse surveillance and necropsy of randomly selected littersmates of the double-KO mice. The loose stools of several double-KO mice were negative for parasites. All mice had free access to laboratory rodent diet (5001, Purina Mills Inc., Richmond, Ind.) and water. This diet contains 23% protein, 45% fat, 6% fiber, and 0.28 ppm selenium as provided by the manufacturer (http://www.labdiet.com).

[0073] Genotyping of Gpx1 and Gpx2-KO mice was done with either Southern or PCR analysis of DNA isolated from tails. For Southern analysis, 10 μg DNA was digested with BamHI or Apal to determine the genotype of Gpx1 and Gpx2, respectively. After overnight digestion, DNA was resolved in 0.75% agarose gel and transferred to Zeta Probe membrane (BioRad Lab., Richmond, Calif.) and probed with [32P]-labeled and random-primed 3 EcoRI fragment of mouse Gpx1 cDNA and mouse Gpx2 exon 2 CDNA. The Southern blot was analyzed by phosphor imaging (Molecular Dynamics, Sunnyvale, Calif.) (13). Polymerase Chain Reactions (PCR) were also performed. The PCR primers for the wildtype Gpx1 allele were mPX101F (DNA SEQ ID NO. 1:5-AAGGAGTGGAGGCGGCTGAGCCG-3') and Gpx15 (SEQ ID NO. 2:5'-ACCGTTACCTGCAGTCTTC3'), which amplified about ~600 bp DNA fragment. The primers for the Gpx1-KO allele were pPNT1pG (SEQ ID NO. 3:5'-CAGTTTCATAGGGCTGAAGACAGAAAT-3') and Gpx15, which amplified ~200 bp DNA fragment. The primers for the wildtype Gpx2 allele were MPX206 (SEQ ID NO. 4:5'-CCCACCTGCTGAGGACTTTA3') and MPXIn9 (SEQ ID NO. 5:5'-TCTATGAGGACTTTA-3'), which amplified ~600 bp DNA fragment. The primers for the Gpx2-KO allele were MPX206 and pPNT1pG, which amplified ~400 bp DNA. Both alleles were amplified in the same reaction tubes.

[0074] Metabolic Studies

[0075] Rectal temperature was measured with Thermalert mouse probe (Model TH-8, Physitemp Instrument Inc., Clifton, N.J.) at the 6-8 am on mice under normal housing. To quantify the food and water consumption and feces and urine output mice were placed in metabolic cages without bedding for 24 h. This setting appeared to be stressful for the double-KO mice, as shown by frequent hunched-over appearance, piloerection of their coat, and loose stools the next day.

[0076] Histology of Small and Large Intestine

[0077] Mice were sacrificed by halothane overdose (Halo- carbon Labs, North Augusta, S.C.). After removing the luminal contents, sections of jejunum, ileum, colon, and rectum were rinsed with phosphate buffered saline, and then fixed in 10% buffered formalin or Bouin’s fixative for 2-3 h. The tissues were then dehydrated in ethanol, and embedded in paraffin and sectioned onto slides. The tissue sections were stained with hematoxylin and eosin (H&E) alone or in addition to periodic acid Schiff (PAS) staining.

[0078] Gpx Activity Assay

[0079] Gpx activity was determined on mouse intestinal and colon epithelium. Jejunal and ileal epithelium were isolated from the proximal and the distal one third of small intestine as described previously. Esworthy, R. S., Mann, J. R., Sam, M. & Chu, F. (2000) Am J Physiol Gastrointest Liver Physiol 279, G426-G436. The Gpx activity was measured with 60 μM H2O2 and 3 mM GSH at pH 7.3. The protein concentration was determined with a BCA assay (Pierce Chemical, Rockford, Ill.) with bovine serum albumin as the standard.

[0080] Transgenic animals having a heterozygous or homozygous disruption in one or more genes can also be crossed to other animals having the same or different homozygous or heterozygous disruptions in the same or different genes to generate numerous combinations of heterozygous and homozygous disruptions of multiple genes, as well known in the art and as demonstrated in the Examples of the present invention.

[0081] Furthermore, a transgenic animal of the invention can be transformed with a heterologous gene of interest having a disruption in order to modulate the expression of the heterologous gene in an animal having a homozygous disruption of the Gpx1 and Gpx2 genes. In this manner, one can determine the effects of modulating the expression of a heterologous gene of interest in a transgenic animal having a homozygous disruption of the Gpx1 and Gpx2 genes.

[0082] As used herein, the term “heterologous gene” or “heterologous nucleic acid sequence” refers to a sequence that originates from a foreign species, or, if from the same species, it may be substantially modified from its original form. The term also encompasses an uncharged nucleic acid sequence that is not normally expressed in a cell. Preferably, the heterologous sequence is operably linked to a promoter, resulting in a chimeric gene. In preferred embodiments, the heterologous gene of interest is associated with either an increase or decrease in at least one sign or symptom of ileitis, colitis, inflammatory bowel disease, ileal cancer and or myeloleukemia. It may also be desirable to observe the effect of a biological response modifier incorporated into the genome of the transgenic animal of the invention. Included in this category are immunopotentiating agents including nucleic acids encoding a number of the cytokines classified as interleukins, interferons, tumor necrosis factor (TNF) tumor suppressor genes, anti-angiogenic genes and the like. See, for example, U.S. Pat. Nos. 6,288,024; 4,879,226; and 6,300,475.
The terms "knockout" and "disruption" each refer to partial or complete reduction of the expression of at least a portion of a nucleic acid or a polypeptide encoded by one or more endogenous genes of a single cell, selected cells, or all of the cells of an animal. The animal may be a "heterozygous knockout" or have a "heterozygous disruption," wherein one allele of one or more endogenous genes have been disrupted. Alternatively, the animal may be a "homozygous knockout" or have a "homozygous disruption," wherein both alleles of one or more endogenous genes have been disrupted.

Methods of generating transgenic mice by inserting a nucleic acid sequence which can cause a disruption in an endogenous gene into the pronuclei of a fertilized mouse oocyte are known in the art (See e.g., U.S. Pat. No. 4,736,866 issued to Leder et al.). Typically, the sequence is inserted into an undifferentiated cell terming an embryonic stem cell (ES cell). ES cells are usually derived from an embryo or blastocyst of the same species as the developing embryo into which it can be introduced. The knockout sequence can cause a disruption in a gene by insertion of an altered nucleic acid sequence into a homologous region of the coding region of the endogenous nucleic acid sequence (usually containing one or more exons) and/or the promoter region of a gene so as to decrease or prevent expression of the full length gene product in the cell. Insertion is usually accomplished by homologous recombination. Such methods are known in the art. By way of example, a disruption construct can be prepared by inserting, for example, a nucleotide sequence comprising an antibiotic resistance gene into a portion of an isolated nucleotide sequence encoding an endogenous gene that is to be disrupted. When this knockout construct is then inserted into an embryonic stem cell, the construct can integrate into the genomic DNA of at least one allele of the gene. Thus, many progeny of the cell will have the gene disrupted and no longer express the nucleic acid or gene or will express it at a decreased level and/or in a truncated form. Also, use of oligonucleotides or antisense nucleic acids which are complementary to at least a portion of a specific mRNA molecule to stall transcription of the mRNA can also be utilized to disrupt gene expression.

**EXAMPLES**

**0085** The invention is further illustrated by the following examples, which are not intended to be limiting.

**Example 1**

**0086** Generation of Double-KO Mice

**0087** Heterozygous Gpx1-KO and Gpx2-KO mice were bred to generate heterozygous double-KO mice. These heterozygous double-KO mice were bred to each other, one sixteenth of the offspring were homozygous double-KO mice. One half of mice were reciprocal homozygous and heterozygous KO’s, so called 3-quarter KO’s. These double-KO and 3-quarter KO were used as breeders to generate the double-KO mice. The genotypes of six mice were analyzed by Southern analysis to examine genetic characteristics of the results of double knockout breeding. Referring now to FIG. 1A, the left panel contains BamHI-digested DNA hybridized with mouse Gpx1 cDNA. The top arrow points at ~11 kb wild type (WT) allele, and the lower arrow points at ~4.3 kb Gpx1-KO allele. The right panel contains Apal-digested DNA hybridized with mouse Gpx2 cDNA. The top arrow points at ~14 kb Gpx2 pseudogene (Ps-Gpx2), the middle arrow points at ~7 kb WT allele, and the lowest arrow points at ~4.9 kb Gpx2-KO allele. The other two DNA fragments of low molecular weights do not correlate with Gpx2 genotypes, and are ignored. The genotypes are shown in the bottom of the panels and are designated as follows: +/-, one wild type and one knockout allele; +/- two wild type alleles; --/-- controls, two knockout alleles.

**0088** Referring now to FIG. 1B, there are shown the results of GPX enzyme activity in the epithelium of mouse lower GI-tract. GPX activity was measured using hydrogen peroxide as the substrate. The error bars represent variances or standard deviations of the means. The number of mice assayed in each group from left to right is 3, 2, 4, 4, and 4 respectively. The genotypes for both Gpx1 and Gpx2 are as in IA.

**0089** The number of the double-KO mice was close to the predicted value from Mendelian genetics. Similar numbers of male and female offspring were obtained. This indicates that the double-KO mice have normal embryonic development and there is no gender bias. Both male and female double-KO mice can be fertile but only a small percentage of mice gain enough weight and appear healthy enough to be used as breeders.

**Example 2**

**0090** Growth of Double-KO Mice

**0091** Referring now to FIG. 2A, there are shown the results of growth activity in adult (45-47 days old) homozygous double-KO mice. There is a graphical representation of the growth rate of a single litter of 8 pups. Male mice are shown in larger symbols, and female mice are shown in smaller symbols. Circles represent Gpx1-/-/Gpx2-/- mice, diamonds represent Gpx1-/-/Gpx2+/- mice, squares represent Gpx1+/-/Gpx2+/- mice, and triangles represent Gpx1-/-/Gpx2+/- mice. The female and male double-KO mice in the top panel started to show growth retardation at 21 and 26 days old.

**0092** Referring now to FIG. 2B, there is shown a graphical representation of the number and age of 33 homozygous double-KO mice at which they first show growth retardation. The double-KO had almost background GPX activity in the mucosa of small and large intestine (Lower Panel of FIG. 1). Since the jejunum mucosa had a high level of GPX-1 and low level of GPX-G1 as shown previously (4), the total GPX activity in this region corresponded only to the Gpx1 gene dosage. The GPX-G1 contributed little to GPX activity in the jejunum even in a homozygous Gpx1-KO background since the heterozygous and homozygous Gpx2-KO mice do not have statistically different GPX activity (P=0.10) as shown in the last two groups in FIG. 1A. A lower level of GPX-1 and a higher level of GPX-G1 are expressed in the ileal mucosa compared with that in jejunal mucosa. The dosage effect of the Gpx2 allele is evident only in the absence of Gpx1 gene expression. In colon mucosa, the heterozygous double-KO has the same level of GPX activity as wildtype mice.

**0093** Gross Phenotypes of Double-KO Mice

**0094** The homozygous double-KO mice had a slower weight gain compared with mice of other genotypes starting around day 16 postnatally. The two double-KO mice had the same birth weight and maintained the same weight gain as their littermates until weaning. Among the 33 double-KO mice followed, 32 showed growth retardation onset at 16-26 days old. The last one started to show growth retardation at 30 days.
Other symptoms often associated with these homozygous double-KO mice include perianal ulceration (redness and irritation of anal region), anal mucous discharge, and diarrhea. One or more of these symptoms occurred as early as 14 days old. However, most of these symptoms were transient except the perianal ulceration, which appeared to be persistent. Older double-KO transgenic mice, over six months old, had a high level of tumor in the ileum.

The younger homozygous double-KO mice had at least 25% mortality. Death or morbidity indicating imminent death occurred between 20-36 days of age. Five of the 33 homozygous mice that we tracked daily died unexpectedly, three more of the 33 mice were terminated when they appeared moribund judging by persistent weight loss, hunched-over posture, or rectal obstruction. No noticeable abnormality was seen in major organs, for example such the liver, kidney, heart, lung, spleen or lymph nodes in the autopsies.

In spite of the severe growth retardation, wasting syndrome, and mortality, the homozygous double-KO mice had similar weight and length of small and large intestine compared with their littersmates up to 25 days old. After 40 days, the length and weight of small intestine in the homozygous double-KO mice began to lag behind their littersmates by 20%. However, the weight of colon and rectum in the homozygous double-KO mice was about 20% heavier than that in their littersmates. This may simply reflect the thickening of colon mucosa in the double-KO mice.

Rectal Hypothermia in Double-KO Mice

To determine if the severe growth retardation was contributed by lack of calorie uptake despite of normal intestinal growth in the homozygous double-KO mice, we monitored the rectal temperature and amount of food uptake by these mice. We found these mice are hypothermic compared with their littersmates either under normal housing condition or in metabolic cages where there was no bedding. Referring now to FIG. 3A, there is a graphical representation of rectal temperatures of double-KO mice as compared to their littersmates. Rectal temperature of homozygous double-KO mice and their littersmates with either combined heterozygous KO or three-quarter KO. Double-KO mice are triangles and their littersmates are squares. The error bars are variances or standard deviations from means of 2-6 mice. Rectal temperatures of the younger (24-36 days old) and more mature (40-67 days old) double-KO mice were 37.0±1.1°C and 35.1±2.2°C respectively. The rectal temperatures of their littersmates were 37.6±0.6°C for all ages under normal housing condition. After being placed in metabolic cages for 24 h, the rectal temperature of 36 day-old double-KO mice had dropped from 36.2±2.5°C to 32.2±1.8°C as shown in FIG. 3B. The control mice did not change their rectal temperature significantly after being housed in metabolic cages. The homozygous double-KO mice (24-49 days old) consumed similar amounts of food (0.16±0.07 g mouse/day weight per day, n=11) as their littersmates (0.10±0.05 g/chow weight body weight per day, n=18). The difference in food intake is not statistically significant. Although the animals had bouts of acute diarrhea and loose stools, they did not have chronic diarrhea.

Referring now to FIG. 3B, there is a graphical representation of hypothermia caused by stress in double-KO mice. Adult (36-day-old) mice were stressed by housing singly or doubly in metabolic cages for 24 hours. The error bars are variances of means from four double-KO mice and six littersmates with Gpx1+/-Gpx2+/- and Gpx1+/-Gpx2+/-genotypes.

Example 4

Inflammation of the Small Intestine and Colon/Rectum

Histological analysis was performed on the cross sections of stomach, jejunum, ileum, colon and rectum after staining with hematoxylin and eosin as shown in FIG. 4. Cross sections from two 20 day-old littersmates with homozygous double-KO and 3-quarter KO genotypes were compared. The 3-quarter KO had apparent normal histology throughout the GI-tract. In contrast, the double-KO mouse had severe ileitis and colitis, although the jejunum and stomach appeared to be unaffected. Crypt abscesses were prevalent in ileum, colon and rectum.

The extent of ileitis and colitis were scored with the histological changes in five categories: (1) severity of the inflammatory cell infiltrate in lamina propria; (2) epithelial cell reactive hyperplasia/atypia; (3) mucin depletion (colon and rectum only); (4) increases in intraepithelial lymphocyte numbers in crypts; and (5) number of inflammatory foci as defined previously (Aranda, R., Sydora, B. C., McAllister, P. L., Binder, S. W., Yang, H. Y., Targan, S. R. & Kronenberg, M. (1997) J. Immunol. 158, 3464-73.) Periodic acid Schiff (PAS) staining was performed on some sections to confirm the depletion of mucin. Referring now to FIG. 4, there are shown the results of histology of mouse ileum, colon and rectum stained with eosin and hematoxylin. One 3-quarter KO (top row) and one homozygous double-KO (lower row) littersmates were sacrificed at 20 days of age. Arrows point at crypt abscesses. The original magnification is 200X.

Table 1 shows the progression of ileitis and colitis from distal to proximal direction in 18 homozygous double-KO mice through early development.

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Age (days)</th>
<th>Total no. of mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jejunum*</td>
<td>11-14</td>
<td>15</td>
</tr>
<tr>
<td>Ileum*</td>
<td>15-17</td>
<td>15</td>
</tr>
<tr>
<td>Proximal colon*</td>
<td>20-27</td>
<td>15</td>
</tr>
<tr>
<td>Distal colon*</td>
<td>15-17</td>
<td>15</td>
</tr>
</tbody>
</table>

*jejunum was sampled at 2-4 cm from stomach; ileum was sampled within 2 cm of cecum; proximal colon was taken within 2 cm of cecum; and distal colon was taken within 1-2 cm from anus.

N = normal (with 0-1 scores, the same as control mice); M = mild colitis (with 4.5-7 scores); S = severe (with 8-10 scores). Scoring was based on the 14 point system described by Aranda et al.[33]: inflammation (0-3 points), mucosal depletion (0-2 points), reactive epithelium (0-3), number of intraepithelial lymphocytes (0-3), inflammatory foci (0-3). No samples were scored in the range of 2-4 or greater than 10.

Spontaneous colitis was shown mostly in the distal colon as early as 11 days of age in 6 out of seven mice analyzed. The proximal colitis was only observed in one of seven 11-14 days old mice analyzed. Most mice of 15 days and older had inflammation in both distal and proximal colon. Ileitis became evident and prevalent in mice of 20-27 days old. No inflammation was seen in the stomach and...
jejunal in all animals up to 60 days old (Table 1 and other observations). Other major organs including heart, liver, lung, kidney, testis, and brain did not have any noticeable abnormality upon gross and histological analysis.

Example 5

[0106] Tumors of the Small Bowel.

[0107] Up to 40% of a generation of homozygous double-KO mice with homozygous disruption of Gpx1 and Gpx2 die before 50 days of age. We have found, however, that up to 60% of Gpx double KO mice can survive up to 13 months of age. Surviving animals were studied to determine whether impaired peroxide detoxification with concomitant distal gastrointestinal inflammation promoted cancer development.

[0108] Many of the mice with homozygous disruption of Gpx1 and Gpx2 exhibited macroscopic tumor located in the distal two-thirds of the small bowel. homozygous double-KO mice having a control mice heterozygous were maintained on a 9% fat chow to prevent wasting and death in the homozygous double-KO mice.

[0109] Homozygous double-KO mice that survived past 50 days of age were euthanized at several predetermined discrete time points or alternatively when distress was evident. Following euthanasia, the GI tracts of the mice were observed macroscopically for the presence of gross tumors and also were processed as Swiss Rolls to survey the entire length of the intestine for histological evidence of dysplasia and inflammation. The tumors were similar in histological appearance to human adenocarcinomas.

[0110] Referring now to FIG. 5, there is shown a graphical representation of the presence of tumors of the small bowel in homozygous double-KO mice having a disruption of the Gpx1 and Gpx2 genes. At five months of age, five of 16 double-KO mice sacrificed had tumors. At 6-8 months, 1 more of 5 additional mice sacrificed had tumors. At 9-13 months, 10 of 21 additional mice sacrificed had tumors. The total number of tumors observed in homozygous double-KO mice having a disruption of the Gpx1 and Gpx2 genes was 16 out of 42 mice that survived to 5 months of age.

[0111] A total of 23 homozygous double-KO mice were examined according to the above procedures. Eleven (11) of the mice had a macroscopic tumor located in the distal two-thirds of the small bowel. Referring now to FIGS. 6 there is shown a graphical representation of the location of tumors and frequency of tumors at precise points along the intestine from the stomach to the cecum. The numbers on the X-axis refer to actual number of mice with tumor at that location. Referring now to FIG. 7, there is shown a photograph depicting two tumors in homozygous double-KO Gpx1/Gpx2 mice and their location the intestine from the stomach to the cecum. Most of these tumors were characterized by a tubular adenomatous pattern that extended over a broad base. In two of the 11 mice showing tumors, neoplastic epithelium had a complex cribriform pattern (back to back glands with no intervening stroma) which was consistent with adenocarcinoma in situ.

[0112] In all mice with tumors, no invasion of the tumors into the serosal adipose tissue was observed. Furthermore, none of the homozygous double-KO mice had tumors in the colon. No tumors were detected using the above methods in 25 age-matched control mice. The control mice were either heterozygous for one or both Gpx1 and Gpx2 or homozygous for one or the other of Gpx1 and Gpx2, i.e., control mice were littermates of the double KO mice that were all other combinations resulting from a cross of homozygous Gpx1 and homozygous Gpx2 knockout mice which were not the double knockouts.

[0113] Mice with disrupted single Gpx1 and Gpx2 genes are apparently normal. This raises some question as to the individual importance of each of these antioxidant enzymes. This lack of an observable deleterious phenotype in single knockout mice also suggests that animals have overlapping defense system against hydroperoxides, since catalase, glutathione S-transferases and AOP-2 can reduce some species of GPX substrates (Jukoby, W. B. (1985) Methods Enzymol. 113:495-9: 35. Kang, S. W., Chae, H. Z., Seo, M. S., Kim, K., Baines, D. L. & Rhee, J. B. (1999) J. Biol. Chem. 274, 6297-302; Fisher, A. B., Dodia, C., Manevich, Y., Chen, J. W. & Feinstein, S. I. (1999) J. Biol. Chem. 274, 21326-34; Esworthy, R., Chu, F. F., and Doroshov, J. H. (1999) in Current Protocols in Toxicology, ed. Maines, M., Costa, L., Reed, D., and Sassa, S. J. (John Wiley & Sons, Inc., pp. 7.11-7.1.32). In contrast to the single knockout mice, the gross abnormality found in mice with combined disruption of Gpx1 and Gpx2 genes demonstrates the uniqueness of GPX activity which cannot be compensated by other types of hydroperoxide-reducing enzymes. This result also suggests that Gpx-1 and Gpx-2 are functionally redundant.

[0114] The GPX-1 appears compensating for lack of GPX-1 in epithelium of small intestine judged by the same level of GPX activity detected in mice expressing 0 and 1 Gpx2 allele. A higher level of GPX-1 in homozygous Gpx2-KO intestine was detected compared to the GPX-1 level in wildtype mice determined by immunoprecipitation (Esworthy et al. (1998) Biochim Biophys Acta 1381,213-26; Esworthy et al. (2000), Am J Physiol Gastrointest Liver Physiol 279, G426-G436). The same level of GPX-GI was detected in Gpx1-KO intestinal mucosa. These observations suggest the Gpx1 gene compensates for lack of Gpx2 gene expression, but not vice versa. The compensation appears to be limited to small intestine but not colon. Alternatively, it is also possible that a part of the expression machinery necessary for selenoproteins in favor of Gpx1 but not Gpx2 gene expression is active in the intestine but not in colon epithelium. This selenoprotein expression machinery includes 3-untranslated region selenocysteine insertion sequence (SECIS) in mRNA (Gasdaska, J. R., Hamey, J. W., Gasdaska, P. Y., Powis, G. & Berry, M. J. (1999) J Biol Chem 274, 25379-25385), selenocystine (RNA(5'-)-(Moustafa, M. E., El-Saadany, M. A., Kandell, K. M., Mansur, D. B. & Lee, B. J., Hattfeld 1998) J. Diamond, A. M. (1998) RNA, 4:1436-43), a SECIS binding protein named SSBP2 (Copeland, P. R. & Driscoll, D. M. (1999) J Biol Chem 274, 25447-54; Copeland, P. R., Fletcher, J. E., Carlson, B. A., Hattfeld, D. L. & Driscoll, D. M. (2000) Embo J 19, 306-314), and mammalian Upl protein (also known as Rent or regulator of nonsense transcripts) (Sun, X., Perllick, H. A., Dietz, H. C. & Masquat, L. E. (1998) Proc. Natl. Acad. Sci. USA 95,10009-14), etc. It is not clear if any of these factors differentiate between Gpx1 and Gpx2 mRNAs. The same GPX level in colon mucosa of wildtype control and heterozygous double-KO mice suggests that this expression machinery for selenoproteins may be a limiting factor.
It is clear that the double-KO mice have almost no GPX activity in the mucosa of distal GI-tract. Although 3-quarter KO mice with no Gpx1 alleles have only a small fraction of total GPX activity in the distal GI-tract, this low level of activity appears to be sufficient to maintain normal physiology. In fact, rodent GI-epithelium may have one-fold higher GPX activity compared with that in humans. The specific activity of GPX in human intestine and colon mucosa is 100-240 mU/mg protein compared with 300-700 mU/mg in rats (4) and mice. Although the difference in GPX activity level in the GI-tract is not as big as that in liver, where humans have 352a±89 mU/mg (Esworthy, R. S., Baker, M. A. & Chu, E. F. (1995) Cancer Res 55, 957-62) and rodents have -4,000 mU/mg (44. Chu, F. F., Esworthy, R. S., Ho, Y. S., Bermeister, M., Swiderk, K. & Elliott, R. W. (1997) Biomed Environ Sci 10, 156-62; Esworthy, R. S., Ho, Y. S. & Chu, F. F. (1997) Arch Biochem Biophys 340, 59-63.), the lower GPX activity level in human GI-tract suggests its higher susceptibility to peroxidative injury.

The first sign of abnormality observed in these double-KO mice is growth retardation. It is well documented that severe Se-deficiency causes growth retardation in young animals (Thompson, K. M., Habach, H., Evenson, J. K. & Sunde, R. A. (1998) J Nutr. 128, 1289-95). Injection of triiodothryonine (T3) to restore plasma thyroid levels in these Se-deficient animals did not increase animal weight gain (Thompson, K. M., Habach, H. & Sunde, R. A. (1995) J. Nutr. 125, 864-73). Since GPXs are Se-dependent enzymes, this slow growth caused by Se-deficiency in 2nd generation rodents can be explained by lack of GPX-1 and GPX-GI in the GI-tract. This suggests that these 2nd generation Se-deficient animals should be examined for colitis. To determine if growth retardation in the homozygous double-KO mice is due to lack of food intake, mice were placed in metabolic cages to monitor the amount of food, water and excretion for a 24-hour period. Often, two mice were placed in one metabolic cage since the double-KO mice could not sustain the stress well when housed alone in this setting. The stress may be contributed by the cooler air due to lack of bedding and shelter. Since the double-KO mice consume the same amount of food as their littermates, and do not have chronic osmotic diarrhea, it is possible that these double-KO mice are either deficient in converting the calorie intake into metabolic fuel as implicated in the older Gpx1-KO mice Espósito, L. A., Kokoszka, J. E., Waymire, K. G., Cottrell, B., MacGregor, G. R. & Wallace, D. C. (2000) Free Radic Biol Med 28, 754-66, or suffering from inflammation-induced cachexia (Liu, Z., Geboes, K., Colpaert, S., Overbeek, L., Mathieu, C., Herve, H., de Boer, M., Boon, L., DHAENS, G., Rutgeerts, P. & Ceuppens, J. L. (2000) J Immunol 164, 6005-14.

Many mammals respond to energy deficit, such as calorie restriction, by lowering body temperature (Lanc, M. A., Baer, D. J., Rumpel, W. V., Weindrich, R., Ingram, D. K., Tilmont, E. M., Cutler, R. G. & Roth, G. S. (1996) Proc Natl Acad Sci USA 93, 4159-64). In fact, fasting can induce torpor or extreme hypothermia in mice (Gavrilova, O., Leon, L. R., Marcus-Samuels, B., Mason, M. M., Castle, A. L., Relefsto, S., Vinson, C. & Reitman, M. L. (1999) Proc Natl Acad Sci USA 96,14623-8). Since these mice have wasting syndrome, we wanted to determine if they also have hypothermia consistent with deprivation in metabolic energy. The hypothermia presented in these mice support the notion that these mice may not be getting enough calories despite unrestricted access to food and normal appetite. It will readily be appreciated by those skilled in the art that determination of hypothermia in mice of the present invention supplied with a high fat diet can be utilized to answer this question.

[0120] Inflammatory Bowel Disease is associated with an increased overall risk for colon and small intestine carcinoma. (Cancer risk is related to disease duration increasing 0.5-1% per year.) Rectal carcinoma risk is increased 2 fold in patients with ulcerative colitis and there is a huge increase in risk for small intestine carcinoma (17 fold) in Crohn’s Disease patients. See e.g., Pohl et al., Hepatogastroenterology, 47(31):57-70 (2000) and Bernstein et al., Cancer 91:854-862 (2001). The appearance of dysplasia over large areas precedes adenoma and carcinoma in both humans and Gpx1/Gpx2 double knockout mice. The Gpx1/Gpx2 double knockout mice are thus a model for the significant risk of small intestine carcinoma associated with Crohn’s disease with regards to precancerous lesions, dysplasia over large areas and dramatic increase in risk for tumors and carcinomas of the small intestine. The Gpx1/Gpx2 double knockout mice of the present invention also develop myeloleukemia and thus present a useful model for this disease also.

[0121] It will be appreciated that the methods and compositions of the instant invention can be incorporated in the form of a variety of embodiments, only a few of which are disclosed herein. It will be apparent to the artisan that other embodiments exist and do not depart from the spirit of the invention. Thus, the described embodiments are illustrative and should not be construed as restrictive.

[0122] References

SEQUENCE LISTING

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<400> SEQUENCE: 1
agggagcgtgc aggcggctgt gaggc

[0158] 36. Gradagni et al., Cancer Res. 61, 2523-2536.
What is claimed is:

1. A transgenic animal whose genome contains a homozygous disruption of the endogenous Gpx1 gene and Gpx2 genes wherein said animal develops cancer.

2. A cell from the transgenic animal of claim 1.

3. The transgenic animal of claim 1 wherein the animal is a mouse.

4. A cell from the transgenic animal of claim 3.

5. A cell of claim 4 which is selected from the group consisting of stem cells, epithelial cells and myeloblasts.

6. The transgenic mouse of claim 3 wherein the genetic background of the mouse is selected from the group consisting of a B6 mouse, a 129SvJ hybrid mouse, a 129S3 hybrid mouse and a ½ B6, ⅔ 129SvJ and ⅔ 129S3 hybrid mouse.

7. A transgenic mouse as in claim 3 which further comprises a mouse which is a germ free mouse.

8. A transgenic animal as in claim 3 wherein the cancer is selected from the group consisting of ileal cancer and myelomarkia.

9. An animal model for cancer which comprises a transgenic animal whose genome comprises a homozygous disruption of the endogenous Gpx1 gene and a homozygous disruption of the endogenous Gpx2 gene and wherein disruption of the Gpx1 and Gpx2 genes is sufficient to effect one or more signs or symptoms in the animal associated with cancer.

10. The model of claim 10 wherein the transgenic animal is a mouse.

11. The model of claim 10 wherein the cancer is ileal cancer.

12. The model of claim 11 wherein the sign or symptom associated with cancer is selected from the group consisting of icitis, colitis, hypothermia, decreased rate of weight gain, perianal ulceration, diarrhea, wasting syndrome, inflammatory bowel disease, dysplasia in the small bowel, one or more tumors in the small bowel.

13. The model of claim 10 wherein the cancer is myelomarkia.

14. The model of claim 10 wherein the genetic background of the mouse is selected from the group consisting of a B6 mouse, a 129SvJ hybrid mouse, a 129S3 hybrid mouse and ¾ B6, ⅔ 129SvJ and ⅔ 129S3 hybrid mouse.

15. A model as in claim 10 wherein the mouse further comprises a mouse which is a germ free mouse.

16. A method to screen for potential therapeutic agents for the treatment of cancer which comprises the steps of:

   a) administering a potential therapeutic agent to a first transgenic animal whose genome comprises a homozygous disruption of both the endogenous Gpx1 gene and
Gpx2 genes and b) maintaining the animal for a time sufficient to permit the detection a change in one or more signs or symptoms in the animal associated with cancer in the transgenic animal;

c) observing the animal for a change in at least one sign or symptom associated with cancer, wherein a second transgenic animal having the same genetic background as the first transgenic animal and whose genome also comprises a homozygous disruption of both the endogenous Gpx1 gene and Gpx2 genes has been maintained under the same conditions as the first animal but has not received the potential therapeutic agent; and

d) determining whether one or more signs or symptoms associated with cancer is present in the second transgenic animal but not in the first transgenic animal; wherein a potential therapeutic agent will be one that causes a lower incidence of at least one sign or symptom associated with cancer in the first transgenic animal.

17. A method as in claim 16 wherein the transgenic animal is a mouse.

18. A method as in claim 17 wherein the cancer is ileal cancer.

19. The model of claim 18 wherein the sign or symptom associated with cancer is selected from the group consisting of ileitis, colitis, hyperthermia, decreased rate of weight gain, perinatal ulceration, diarrhea, wasting syndrome, inflammatory bowel disease, dysplasia in the small bowel, one or more tumors in the small bowel.

20. The method of claim 17 wherein the cancer is myeloleukemia.

21. A method as in claim 17 wherein the genetic background of the mouse is selected from the group consisting of a B6 mouse, a 129Sv/J hybrid mouse, a 129S3 hybrid mouse and a ½ B6, ¼ 129Sv/J and ¼ 129S3 hybrid mouse.

22. A method as in claim 17 wherein the mouse further comprises a mouse which is a germ free mouse.

23. A method as in claim 17 wherein determination of whether one or more signs or symptoms associated with cancer is present in the second mouse but not in the first mouse comprises sacrificing the first and second mouse after a time sufficient for the detection of at least one sign or symptom associated with cancer in the first mouse and second mouse has elapse.

24. The method as in claim 17 wherein the determination of whether one or more signs or symptoms associated with cancer is present in the second mouse but not in the first mouse comprises withdrawing a body fluid or other bodily substance from the first and second mouse and analyzing the body fluid for the presence of one or more signs or symptoms associated with cancer is present.

25. The method as in claim 24 wherein the bodily fluid is selected from the group consisting of blood and stool.

26. A transgenic animal whose genome comprises a homozygous disruption of both the endogenous Gpx1 gene and Gpx2 genes wherein the animal’s genome additionally comprises a DNA sequence encoding a heterologous gene of interest.

27. A transgenic animal as in claim 26 which is a mouse.

28. The mouse of claim 27 wherein the heterologous gene of interest is selected from the group consisting of an antiangiogenic protein, an immunomodulator, a ribozyme, a peptide and an antisense nucleic acid.

29. A transgenic mouse as in claim 29 wherein the mouse is a mouse that is selected from the group consisting of a B6 mouse, a C57B16/J hybrid, a 129Sv/J hybrid, al29S3 hybrid and ¼ B6, ¼ 129Sv/J and ¼ 129S3 hybrid.

30. A transgenic mouse as in claim 29 wherein the hybrid mouse is ½ B6, ¼ 129Sv/J and ¼ 129S3.

31. A method for assessing the therapeutic effect of a heterologous gene of interest on the development of cancer which comprises the steps of:

a) expressing the heterologous gene of interest in a first transgenic animal whose genome comprises a homozygous disruption of both the endogenous Gpx1 gene and Gpx2 genes, and

b) maintaining the first transgenic animal for a time sufficient to permit the detection a change in one or more signs or symptoms in the first transgenic animal associated with cancer in the first transgenic animal;

c) observing the first transgenic animal for a change in at least one sign or symptom associated with cancer, wherein a second transgenic animal having the same genetic background as the first transgenic animal and comprising a homozygous disruption of both the endogenous Gpx1 gene and Gpx2 genes does not express the gene of interest, wherein the second transgenic animal has been maintained under the same conditions as the first transgenic animal; and

d) determining whether one or more signs or symptoms associated with cancer is present in either the second animal, wherein a gene of interest which reduces cancer will be one that causes a lower incidence of at least one sign or symptom associated with cancer in the first animal.

32. A method as in claim 31 wherein the transgenic animal is a mouse.

33. A method as in claim 32 wherein the mouse is a mouse that is selected from the group consisting of a B6 mouse, a C57B16/J hybrid, a 129Sv/J hybrid, al29S3 hybrid and ½ B6, ¼ 129Sv/J and ¼ 129S3 hybrid.

34. A transgenic mouse as in claim 33 wherein the hybrid mouse is ⅔ B6, ¼ 129Sv/J and ¼ 129S3.

35. A method of identifying markers associated with cancer, the method comprising: comparing the presence, absence or level of expression of at least one gene or protein in a transgenic animal whose genome comprises a homozygous disruption of both the endogenous Gpx1 gene and Gpx2 genes with the level or expression of the gene or protein in a second animal, wherein the second animal has the same genetic background as the first animal but does not comprise a homozygous disruption of both the endogenous Gpx1 gene and Gpx2 genes, wherein the difference between the transgenic animal and the second animal in the presence, absence or level of expression of the gene or protein indicates that the expression of the gene is a marker associated with cancer.

36. A method as in claim 34 wherein the transgenic animal is a mouse.

37. A method as in claim 36 wherein the mouse is a mouse that is selected from the group consisting of a B6 mouse, a C57B16/J hybrid, a 129Sv/J hybrid, al29S3 hybrid and ½ B6, ¼ 129Sv/J and ¼ 129S3 hybrid.

38. A transgenic mouse as in claim 37 wherein the hybrid mouse is ⅔ B6, ¼ 129Sv/J and ¼ 129S3.
39. A method as in claim 35 wherein the gene or protein is selected from the group consisting of telomerase and mucin antigens.

40. A transgenic double knockout mouse whose genome comprises a homozygous disruption of the endogenous Gpx1 gene and a homozygous disruption of the endogenous Gpx2 gene, wherein each disruption comprises the insertion of a transgene, and wherein the combined disruptions result in a decreased level of GPX-1 and GPX-GI production and decreased number of cells producing GPX-1 and GPX-GI in the transgenic mouse as compared to a nontransgenic mouse.

41. A transgenic double knockout mouse as in claim 40 which exhibits one or more physiological symptoms selected from the group consisting of ileitis, colitis, hypothermia, decreased rate of weight gain, perianal ulceration, diarrhea, wasting syndrome, inflammatory bowel disease and cancer of the lower gastrointestinal tract.

42. A cell isolated from a double knockout mouse as in claim 40.

43. A cell as in claim 42, selected from the group consisting of a stem cell, an epithelial cell and a myofibroblast.

44. A cell as in claim 43 which is a stem cell.

45. A cell as in claim 43 which is an epithelial cell.

46. A cell as in claim 43 which is a myofibroblast.

47. A transgenic double knockout mouse as in claim 40 which further comprises a mouse which is a germ free mouse.

48. A transgenic double knockout mouse as in claim 1 wherein said knockout mouse is a mouse with a B6 genetic background.

49. A transgenic double knockout mouse as in claim 1 wherein said knockout mouse is a mouse with a hybrid mouse having a ½ B6, ¼ 129 S1J and ¼ 129 S3 genetic background.

50. A method of selecting an agent for treating a metabolic disorder comprising:

(a) measuring at least one symptom selected from the group consisting of ileitis, colitis, hypothermia, decreased rate of weight gain, perianal ulceration, diarrhea, wasting syndrome, inflammatory bowel disease and cancer of the lower gastrointestinal tract in a knockout mouse whose genome has been manipulated to comprise a homozygous disruption of both the endogenous Gpx1 gene and Gpx2 genes, wherein the disruption of both the Gpx1 gene and Gpx2 genes results in said knockout mouse exhibiting one or more of said diseases, symptom or symptoms; (b) administering an agent to said mouse; (c) measuring one or more of said symptoms in the mouse after administering the agent; and (d) comparing at least one of said disease, symptom or symptoms in the mouse before and after administering the agent, wherein a decrease in at least one of said diseases, symptom or symptoms after administering the agent indicates the agent is an agent for treating said disease, symptom or symptoms.

51. A method as in claim 50 wherein said knockout mouse is a mouse with a B6 genetic background.

52. A method of selecting an agent that modulates GPX enzyme activity comprising:

(a) administering an agent to a first group of isolated mouse cells and not to a second group of mouse cells, wherein the genomes of both the first and second isolated mouse cell groups have been manipulated to comprise a homozygous disruption of both the endogenous Gpx1 gene and Gpx2 genes, and wherein the disruption of both the Gpx1 gene and Gpx2 genes prevents expression of functional GPX-1 and GPX-GI proteins; and (b) determining the amount of GPX enzyme activity of the first and second cell groups, wherein a difference in the amount of proliferation of the first cell group as compared to the second cell group indicates that the agent modulates GPX enzyme activity.

53. The method of claim 52 wherein the mouse cells are selected from the group of cell types consisting of stem cells, epithelial cells, intestinal epithelial cells and myofibroblast cells.

54. The method of claim 53 wherein the mouse cells are epithelial cells.

55. The method of claim 54 wherein the epithelial cells are intestinal epithelial cells.

56. The method of claim 53 wherein the cells are stem cells.

57. The method of claim 53 wherein the cells are myofibroblasts.

58. A method of selecting an agent for treating a metabolic disorder comprising:

(a) measuring at least one symptom in a first double knockout mouse having a first genetic background, whose genome is manipulated to comprise a homozygous disruption of both the endogenous Gpx1 and Gpx2 genes, wherein the disruption of both the Gpx1 and Gpx2 genes results in said knockout mouse exhibiting a disease, symptom or symptoms selected from the group consisting of: ileitis, colitis, hypothermia, decreased rate of weight gain, perianal ulceration, diarrhea, wasting syndrome, inflammatory bowel disease and cancer of the lower gastrointestinal tract; (b) measuring said symptom in a second double knockout mouse having a second genetic background, whose genome is manipulated to comprise a homozygous disruption of both the endogenous Gpx1 and Gpx2 genes, wherein the disruption of both the Gpx1 and Gpx2 genes results in said knockout mouse exhibiting at least one of said disease, symptom or symptoms; (c) administering an agent to said first and second mouse; (d) measuring one or more of said symptoms in the first and second mouse after administering the agent; and (e) comparing at least one of said symptoms in said first and second mouse before and after administering the agent, wherein a decrease in said disease, symptom or symptoms after administering the agent indicates the agent is an agent for treating said disease, symptom or symptoms associated with a metabolic disorder.

59. The method of claim 57 wherein one of said first and second mouse has a B6 genetic background.

60. A transgenic mouse which has a homozygous knockout of the Gpx1 gene and a heterozygous knockout of one allele of the Gpx2 gene.
61. An animal model for the study of the degree of functional redundancy of GPX-1 and GPX-GI in the ileum and colon comprising the mouse of claim 60.

62. A transgenic mouse which has a homozygous knock-out of the Gpx2 gene and a heterozygous knockout of one allele of the Gpx1 gene.

63. An animal model for the study of the degree of functional redundancy of GPX-1 and GPX-GI in the ileum and colon comprising the mouse of claim 62.

64. Isolated mammalian cells comprising a diploid genome including chromosomally incorporated transgenes, wherein the transgenes disrupt both alleles of the genomic Gpx1 gene and Gpx2 genes and inhibit expression of said genes.

65. The method of claim 57 wherein one of said first and second mouse has a ½ B6, ¼ 129 SvJ and ¼ 129 S3 background.

66. A method of selecting an agent for treating a metabolic disorder comprising:

(a) measuring at least one symptom selected from the group consisting of ileitis, colitis, hypothermia, decreased rate of weight gain, perianal ulceration, diarrhea, wasting syndrome, inflammatory bowel disease and cancer of the lower gastro-intestinal tract in a knockout cell whose genome has been manipulated to comprise a homozygous disruption of both the endogenous Gpx1 gene and Gpx2 genes, wherein the disruption of both the Gpx1 gene and Gpx2 genes results in said knockout mouse exhibiting one or more of said diseases, symptom or symptoms;

(b) administering an agent to said cell;

(c) measuring one or more of said symptoms in the cell after administering the agent; and

(d) comparing at least one of said disease, symptom or symptoms in the cell before and after administering the agent, wherein a decrease in at least one of said diseases, symptom or symptoms after administering the agent indicates the agent is an agent for treating said disease, symptom or symptoms.